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1/00907 A1

(54) Title: METHOD FOR STABLE CHROMOSOMAL MULTI-COPY INTEGRATION OF GENES

(57) Abstract: The present invention solves the problem of integrating multiple copies of a gene of interest by homologous recombination into well defined positions adjacent to conditionally essential genes in a bacterial host strain chromosome, which already comprises at least one copy of the gene of interest in a different position.

10022.204-WO.ST25 SEQUENCE LISTING

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 Andersen, Jens Tønne
 Olsen, Carsten

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10022.204-WO.ST25

tttctaccat ccttgactgt acaggtagca atggcaggtg ccatgttgat tggtctgcat 6780 catcgcatct gttatacgac gagcgcttcg gtcttaactg aagcagttaa gcaatca 6837 <210> 50

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<212> DNA

<213> Artificial sequence: repF expression cassette

<400> 50 gaattccggc ccaacgatgg ctgatttccg ggttgacggc cggcggaacc aaggggtgat 60 cggtcggcgg aaatgaaggc ctgcggcgag tgcgggcctt ctgttttgag gattataatc 120 agagtatatt gaaagtttcg cgatcttttc gtataattgt tttaggcata gtgcaatcga 180 taagcttgaa ttcggaggcc gttattatat catgagcgaa aatgtaataa aagaaactga 240 aaacaagaaa aattcaagag gacgtaattg gacatttgtt ttatatccag aatcagcaaa 300 agccgagtgg ttagagtatt taaaagagtt acacattcaa tttgtagtgt ctccattaca 360 tgatagggat actgatacag aaggtaggat gaaaaaagag cattatcata ttctagtgat 420 gtatgagggt aataaatctt atgaacagat aaaaataatt acagaagaat tgaatgcgac 480 tattccgcag attgcaggaa gtgtgaaagg tcttgtgaga tatatgcttc acatggacga 540 tcctaataaa tttaaatatc aaaaagaaga tatgatagtt tatggcggtg tagatgttga 600 tgaattatta aagaaaacaa caacagatag atataaatta attaaagaaa tgattgagtt 660 tattgatgaa caaggaatcg tagaatttaa gagtttaatg gattatgcaa tgaagtttaa 720 atttgatgat tggttcccgc ttttatgtga taactcggcg tatgttattc aagaatatat 780 aaaatcaaat cggtataaat ctgaccgata gggatcc 817

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 01/00436 A. CLASSIFICATION OF SUBJECT MATTER IPC7: C12N 15/90, C12N 15/68, C12N 15/67 // C12N 15/75 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC7: C12N Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched SE,DK,FI,NO classes as above Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. WO 9941358 A1 (NOVO NORDISK A/S), 19 August 1999 1-66 (19.08,99)EP 0185512 A1 (NOVO INDUSTRI A/S), 25 June 1986 Α 1-66 (25.06.86)A EP 0972838 A1 (ROCHE DIAGNOSTICS GMBH), 1-66 19 January 2000 (19.01.00) Further documents are listed in the continuation of Box C. See patent family annex. Special categories of cited documents: later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be special reason (as specified) considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document referring to an oral disclosure, use, exhibition or other document published prior to the international filing date but later than "&" document member of the same patent family the priority date claimed Date of the actual completion of the international search Date of mailing of the international search report 2 7 -11- 2001 <u> 23 November 2001</u> Name and mailing address of the ISA/ Authorized officer Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Yvonne Siösteen/EÖ

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INTERNATIONAL SEARCH REPORT

Information on patent family members

06/11/01 PCT/

International application No.
PCT/DK 01/00436

	ent document n search report		Publication date		Patent family member(s)	Publication date
MO	9941358	A1	19/08/99	· AU	2610399 A	30/08/99
				CN	1272135 T	01/11/00
	•			EP	1062318 A	27/12/00
				US	6100063 A	08/08/00
EP	0185512	A1	25/06/86	SE	0185512 T3	
				AT	55413 T	15/08/90
				ΑU	592542 B	18/01/90
				AU	5108185 A	19/06/86
				CA	1292197 A	19/11/91
				DE	3579133 D	00/00/00
				DK	159282 B,C	24/09/90
				DK	571985 A	13/06/86
				DK	594084 A	13/06/86
				FI	85876 B,C	28/02/92
				FI	854893 A	13/06/86
				JP	2571338 B	16/01/97
				JP	2576970 B	29/01/97
				JP	6086669 A	29/03/94
				JP	61139392 A	26/06/86
				МО	172063 B,C	22/02/93
				NO	854966 A	13/06/86
				US	4920048 A	24/04/90
EP	0972838	A1	19/01/00	AU	720627 B	08/06/00
				AU	4011599 A	10/02/00
				BR	9902792 A	09/05/00
				JP	2000050888 A	22/02/00
				TR	9901650 A	00/00/00
				US	6291245 B	18/09/01